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In vitro management of the early blight of tomato by various chemical Fungicides and plant products

Muhammad Saqib Ajmal[1], Ifrah Rashid[2] and Rabia Tahir Bajwa[*3]

[1]Department of Entomology, The Islamia University of Bahawalpur, Pakistan; [2]Department of Plant Pathology, MNS-University of Agriculture, Multan Pakistan; [3]Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan
Corresponding author's e-mail: rabia.kpr@gmail.com

Pathogens reduce crop yield and quality, making their control necessary. In vitro studies examined the effects of botanicals (marigold, kikar, and ginger) and fungicides (Antracol, Captan, and Dithane M 45) against *Alternaria solani*. Botanicals (marigold, kikkar and ginger), fungicides (Antracol, Captan, and Dithane M 45), concentrations of botanicals (50%, 75% and 100%) and concentrations of fungicides (250 ppm, 500 ppm, and 1000 ppm) were the factors studied to effect the inhibition of *A. solani*. Mycelial growth diameter, inhibition, and spore germination were measured. Botanicals displayed considerable anti-fungal activity against *A. solani*, which increased with concentration. All tested botanicals and fungicides inhibited *A. solani*, reduced mycelial growth, and reduced spore germination. Ginger and Antracol have shown the strongest effects against *A. solani* at each concentration. Kikkar showed the minimum inhibition of *A. solani*. These botanicals can suppress pathogenic fungi as natural fungicides. Further research on botanicals should pinpoint the active compounds that cause this anti-fungal activity and test their anti-fungal activity on other fungi at different concentrations. These plant extracts may also control early blight disease in field experiments.

Keywords: Fungal blight, *Alternaria solani*, chemical management, plant extracts.

INTRODUCTION

The tomato (*Solanum lycopersicum* Linnaeus) is the second-largest non-grain food crop after potatoes (Gondal *et al*., 2019). It is a major element in diets worldwide and essential to many meals. The popular, horticultural crop tomato originated in the Andes of South America. China dominates worldwide tomato production with 56,423,811 tonnes of annual production, followed by India with 18,399,000 tonnes. The global tomato production stands at 177,118,248 tons. Pakistan harvests 575,923 tonnes of tomatoes on 60,307 hectares (FAO, 2021). In Pakistan, Sindh produces the most tomatoes on 279 thousand hectares, or 43.6% of Sindh acreage, followed by Khyber Pakhtunkhwa, Baluchistan, and Punjab. In 2017, Baluchistan produced 20.8% of Pakistan's total tomato acreage and 24.6% of its output.

Around 20,000 fungal plant pathogens cause 85% of plant diseases (Ong, 2011). *A. solani* causes a catastrophic disease that annually decreases tomato quality and productivity (Rashmi and Vishunavat, 2012). Tomato plants have long struggled with soil and air pathogens, causing annual crop losses and financial stress for farmers. Early blight, caused by Alternaria species, is extremely destructive to tomatoes and other solanaceous crops. It loses tonnes of annual production and 79% of global agricultural output (Gomes *et al*., 2010). The disease can attack any part of a tomato plant above ground at any stage (Akhtar *et al*., 2019; Mphahlele *et al*., 2020). Early blight in tomatoes and potatoes is caused by the soil-borne pathogen Alternaria solani (Agrios, 2005), infecting brassicae, cucurbits, citrus, crucifers, and alfalfa. This pathogen destroys leaves, stems, fruits, and stalks. Black concentric rings and 1.5-centimeter round lesions are leaf indicators. Leaf blight usually starts on older or lower leaves and moves up the plant. Falling diseased leaves damage agricultural productivity (Adhikari *et al*., 2017; Mahawar *et al*., 2020).

Fungicide treatment reduces early blight severity most effectively and is the most commonly used control method. Fungicide application is an effective, fast technique to inhibit the fungal growth that causes many significant plant

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infections. Many fungicides can treat A. solani and other infections (da Silva *et al*., 2012). Several studies have determined the most effective systemic and contact fungicides. Pathogen spores may be resistant to fungicides that limit penetration and germination. Using fungicides from different groups at the right intervals and concentrations prevents resistance. Novel fungicides, especially high-risk single-site fungicides, necessitate anti-resistance measures. Multi-site protectants like chlorothalonil and mancozeb may delay single-site fungicide resistance (Ashour and Ahmed, 2012; Chourasiya *et al*., 2013).

The control of foliar diseases may benefit from using secondary metabolites found in plants. Botanicals with anti-fungal properties have recently received much interest. Many plant pathogenic fungi are being combated today with the help of plants. Plants have commercial and environmental benefits as biocontrol agents (Swami and Alane , 2013). Plant-based natural chemicals are more cost-effective than synthetic fungicides for small-scale farmers with limited financial resources and are widely accessible in developing countries. Several studies have shown that different plant extracts with anti-fungal efficacy against *A. solani* effectively kill microorganisms. Many studies have shown the efficacy of botanicals as an effective bio-control for various fungal plant diseases, including *Alternaria solani* (Shafique *et al*., 2011; Mudyiwa *et al*., 2016). Due to the non-hazardous effects of biocontrol, many scientists recommend using botanicals.

Timely control becomes necessary as the early tomato blight disease is increasing daily and destroying crop productivity. The current study aims to assess the efficacy of several fungicides and botanicals against tomato early blight disease and their comparison.

MATERIALS AND METHODS

***Sample Collection and Isolation*:** Tomato leaves showing early blight symptoms were collected from vegetable fields near Karachi Mor Bypass Bahawalpur. The diseased samples were processed in Plant Pathology Laboratory at the Islamia University of Bahawalpur. After surface sterilization with 70% ethanol for 60 seconds, the 5 mm discs of the diseased sample and the adjacent small symptomless part were cultured on potato dextrose agar (PDA) media and incubated at 27°C for four to five days. The culture was purified through the single hyphal technique (Tutte, 1969). The purified culture was multiplied and preserved on PDA slants at 4°C.

***Preparation of Plant crude extract*:** The leaves of kikkar (*Acacia nilotica*) and marigold (*Tagetes erecta*) were collected from different areas of Bahawalpur and the ginger (*Zingiber officinale*) bulbs were obtained from the local market. Following a thorough washing in water and surface sterilization with 2% sodium hypochlorite (NaOCl) for 30–60 seconds, 50 g of plant material from each species was crushed in a mortar and pestle with sterile distilled water added at a rate of 10 ml/10 g of plant tissue (Nashwa and Abo-Elyousr, 2012). The resulting homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant solutions were collected. Whatman No. 1 filter paper was used to filter the supernatant, which was then heated to 120 °C for 30 minutes to kill any contamination. Using the 100% crude extract from the various plant sources, 50% and 75% dilutions were made. According to Mohana and Raveesha (2007), the obtained extracts were used as the crude extract, which is the 100% concentration. Concentrates were preserved at 4 °C for later use.

***In vitro management of A. solani through fungicides*:** Four different fungicides, viz., Antracol 70 WP, Dithane M 45 WP 80 and Captan, were acquired from a local store so that their effects on the mycelial diameter expansion of *A. solani* could be measured using the poisoned food method. In sterile molten PDA media, three different fungicides were distributed at random: Antracol (70 % ai /kg Propineb), Dithane M 45 (80 % wt Zinc Manganese Ethylenebis Dithiocarbamate), and Captan (80 % wt % Captan). Fungicides of 0.25 mg, 0.5 mg, and 1 mg were dissolved in SDW to produce 250 ppm, 500 ppm, and 1000 ppm, respectively. Five days were spent in a 25±2 °C incubator with the petri dishes. The percentage of effective fungicides was determined by measuring the diameter at which the targeted pathogen grew compared to the control.

***Mycelia Diameter Measurement for Pathogen Detection*:** After that, five ml of 50%, 75%, and 100% natural concentrate of kikkar, marigold, and ginger were added to the cooled liquid PDA in the Petri dishes. One ml of 70% ethanol (the positive control) was placed on each Petri plate using an inoculating needle. Once the PDA medium had hardened completely, a five-millimeter disc containing a 72-hour-old A. solani culture was inoculated into PDA in the middle of the Petri plates. Incubation at 25±2 was applied to the plates. For comparison, a set of Petri dishes containing the same volume of distilled water but no extract was considered the control. The mycelium diameter expansion of *A. solani* was measured by laying a string perpendicular to a ruler that was 30 centimeters in length. The median diameter was determined for each plant species and fungicide at each concentration. The data was recorded on the 3rd, 5th, and 7th days of inoculation.

***Determination of Mycelial Inhibition Percentage*:** Colony diameter was measured millimetres after incubation, as described by Singh and Tripathi (1999). There were three replications of each treatment. In order to determine the extent to which the extracts inhibited mycelial growth, we used the following formula:

$$\text{Mycelium Inhibition Percentage} = \frac{Mc - Mt}{Mc} \times 100$$

Where Mc represents the diameter of mycelium growth in control and Mt represents the diameter of mycelium growth in treatment.

***Assessment of Spore Germination*:** One disc (1 cm) was extracted from each Petri plate containing a seven-day-old culture of A. solani, and the conidia were counted using a hemocytometer. In preparation for spore collection, two millilitres of distilled water were used to wash the disc (1 cm in diameter). Spores were counted by placing a single drop of solution on a hemocytometer. The formula used to calculate the success rate was as follows:

$$\text{Spore germination (\%)} = \frac{No.of\ germinated\ spores}{Total\ no.of\ examined\ spores} \times 100$$

***Statistical Analysis*:** An analysis of variance (ANOVA) and a least significant difference (LSD) test were applied to data gathered from in vitro anti-fungal experiments. SAS/STAT Software for Windows was used for all the statistical analyses.

RESULTS

***Effect of botanicals and fungicides on the mycelial growth of A. solani*:** The diameter of growing *A. solani* mycelia varied with plant species, fungicides, and their concentrations. When the concentration of the various plant extracts and fungicides increased, the width of the mycelia colony was minimized. The mycelial growth diameter was measured and found to be the greatest (60 mm) for control and the least (7.25 mm) for ginger at the 100% concentration level. For all three concentrations tested (50%, 75%, and 100%), ginger caused the greatest reduction in *A. solani* colony diameter. However, its effect was not substantially different from marigolds at 250 ppm, 500 ppm, and 1000 ppm.

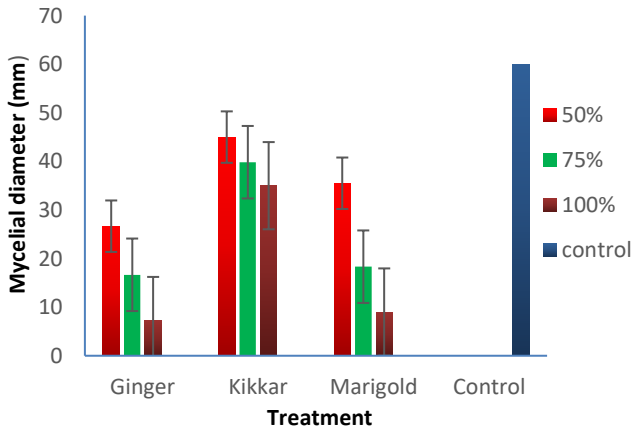

Figure 1. Effect of botanicals on mycelial growth of *A. solani* at different concentrations.

***Effect of botanicals and fungicides on inhibition percent*:** The percentage of inhibition was affected varyingly by different plant types and concentrations. The percentage of *A. solani* inhibited by the plant extracts and fungicides rose as their concentration rose. When applied at maximum concentration, ginger showed the highest percentage of inhibition (87%) out of all applied treatments. This was followed by marigold at 1000 ppm with an inhibition percentage of 85.9%, Antracol at 1000 ppm with an inhibition percentage of 79.45%, Dithane M-45 at 100% concentration with an inhibition percentage of 66.66%, Captan at 1000 ppm with a 59.45% inhibition percentage, and kikkar at 100% concentration with a 41.66% inhibition percentage.

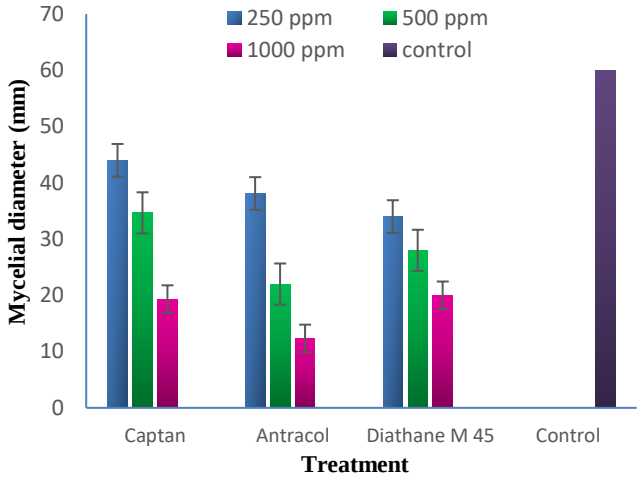

Figure 2. Effect of fungicides on mycelial growth of *A. solani* at different concentrations.

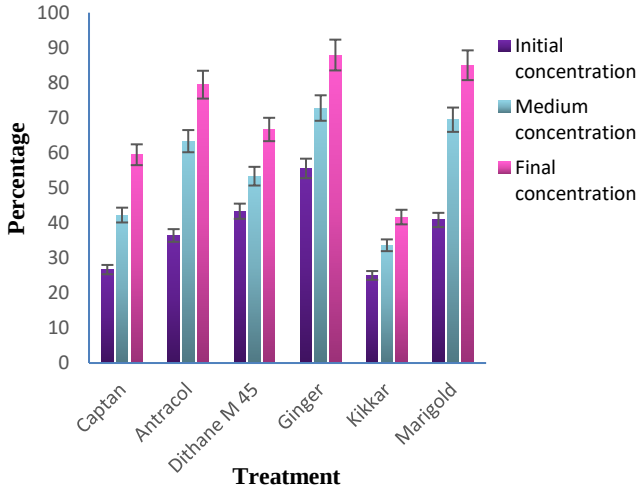

Figure 3. Inhibition percentage of all tested botanicals and fungicides on *A. solani*.

***Effect of botanicals on spore germination*:** The germination rate of *A. solani* spores was shown to be dose-dependent, meaning that treatments (plant extracts and fungicides) had different effects at different concentrations. When the concentration of the plant extracts or fungicides varied, so did the percentage of germinating spores. The percentage of spores that germinated after being exposed to ginger was

found to be considerably ($P < 0.05$) higher than before exposure, while the percentage of spores that germinated after being exposed to marigold was found to be the highest for all three concentration conditions.

DISCUSSION

Our findings show that the plant extracts we tested (ginger, marigold, and kikkar) have anti-fungal properties and can inhibit the growth of A. solani. The concentration of the extracts had a linear relationship with the extent to which mycelia development was inhibited. This is consistent with the in-vitro action experiments on certain plant extracts against wheat seed-borne pathogens like *Aspergillus* spp. (Hasan *et al*., 2005). For example, Swami and Alane (2013) discovered that plant extracts effectively prevented mungbean seeds from becoming infected with fungal pathogens at the highest concentrations they tested. The current findings are consistent with those of other researchers who found that plant extracts, such as those from ginger (*Zinger officinale*) (Fawzi *et al*., 2009), inhibit mycelial development. The tested fungicides (Antracol, Dithane M 45 and Captan) can also potentially control *A. solani* effectively. The chemicals may involve in the cell wall degradation of the pathogen. Various studies have shown the efficiency of Captan (Ganeshan *et al*., 2009), Dithane M45 against *A. solani* and the results are in line with the current findings.

Compared with the control, the mycelial diameter of *A. solani* was reduced by a mean of 33.34 mm at 50%, cm at 42.44 mm at 75%, and 52.75 mm at 100% after exposure to ginger. Over 400 different compounds, including volatile and non-volatile chemical constituents such as zingerone, shogaols and gingerols, sesquiterpenoids (-sesquiphellandrene, bisabolene, and farnesene), and a small monoterpenoid fraction (-phelladrene, cineol, and citral) contribute to ginger potent inhibition potential (Chrubasik *et al*., 2005). Diallyl monosulfide, diallyl disulfide (DADS), diallyl trisulfide found in ginger were effective against A. solani. Tagetes lucida methanol extracts were shown to impede 89% of *F. moniliforme* colony radial expansion, according to research by Céspedes *et al*. (2006). Anti-fungal compounds, such as thiophenes, may be responsible for the action of the marigold extract on the growth of *F. verticilloides* mycelium; the antimicrobial efficacy of this compound has been validated by multiple investigations, as stated by multiple authors (Ali *et al*., 2020). The thiophene-rich extracts from marigold roots and the essential oil from the leaves have been shown in another investigation to have effective anti-fungal action against various soil-borne and foliar plant diseases (Saha *et al*., 2012). The findings of this study are consistent with marigold is efficient against *F. oxysporum* since it limits the clinical symptoms induced by the pathogen by 88.5%. Some anti-fungal chemicals, such as sesquiterpenes, saponins, and flavonoids, are in marigolds at absolutely high concentrations. Other studies have found that kikkar has anti-fungal activity against *A. solani* (More and Baig, 2017).

Gingerols and shogals of ginger are less likely to be lost to diffusion during plant extract processing than garlic and onion alliin. The variety of plant extract and its concentration significantly affected the spore germination rate. The percentage of viable spores germinating was proportionally lower as the quantity of plant extracts was raised. The lowest spore growth rate (19%) was achieved with ginger at 100% concentration. Fawzi *et al*. (2009) found that plant extracts such as cinnamon (*Cinnamomum zeylanicum*), laurel (*Laurus nobilis*), and ginger (*Zinger officinale*) exhibited potent anti-fungal activity, demonstrating significant inhibition of the growth of *Alternaria alternata* and *Fusarium oxysporum*. These results are consistent with those obtained here, demonstrating the efficacy of ginger as a biocontrol. Our results are consistent with those of Fawzi *et al*. (2009) research that found ginger to be the most effective in suppressing fungal development. While all tested botanicals and fungicides were useful, ginger and marigold were the most effective at inhibiting *A. solani*. This is consistent with the findings of Islam and Faruq (2012), who discovered that garlic clove and ginger rhizome were effective in suppressing the fungi responsible for damping off disease, *F. oxysporum* and *Scleretonium rolfsii*.

***Conclusions and future recommendations*:** Plant extracts can be a safe alternative to toxic fungicides for *A. solani* management. Our research indicates that the anti-fungal characteristics of plant extracts from kikkar, ginger, and marigold; and fungicides, i.e., Dithane M45, Captan, and antracol, make them suitable for use in biocontrol of A. solani. Tested fungicides and plant extracts both inhibit A. solani growth. There is conclusive evidence that all tested treatments can significantly inhibit fungal growth and decrease mycelial production in A. solani. Ginger was the most efficient plant extract, followed by marigold, Antracol, Diathane, Captan and Kikkar. It's also possible that higher concentrations of plant extracts and fungicides would be optimal for inhibiting fungal development. We suggest that more studies be conducted on plant extracts to determine which compounds within the extracts are responsible for their fungicidal effects. It is also suggested that further research be conducted to determine the anti-fungal activity of the examined plant extracts against additional fungi at varying concentrations. The effectiveness of these plant extracts in preventing *A. solani* caused diseases like early blight may be tested in subsequent field trials.

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